

## Research Note

# Enumeration of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* in Broiler Carcass Rinses before and after Simulated Transport in Artificial Ice for 24 Hours

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## ABSTRACT

The maintenance and survival of target pathogens during transport from the field collection site to the analytical laboratory is essential for obtaining accurate and reliable data. This study was conducted to compare the efficacy of sterile tap water (SW), buffered peptone water (BPW), and universal preenrichment broth (UP) for maintaining populations of *Campylobacter* spp., *Salmonella*, and *Escherichia coli* for 24 h under simulated transport conditions. Freshly processed broiler carcasses ( $n = 100$ ) were rinsed in SW. The rinses were divided, and components were added to create equal volumes of rinse samples consisting of SW, BPW, and UP. The rinses were analyzed for the target organisms immediately and again after 24 h of simulated chilled transport conditions. The only meaningful difference between the different transport media was found for UP, which recovered fewer *E. coli* than did either SW or BPW. These findings support the conclusion that either SW or BPW should be used as a broiler carcass rinse and/or transport medium to accurately depict the levels or presence of these three target bacteria as a whole. Because potable water differs in pH and hardness across the United States, a follow-up study was conducted to investigate whether water hardness or pH within the ranges normally found across the United States would affect *Campylobacter* recovery from carcass rinses. No significant differences were detected.

Several human pathogens frequently are associated with raw poultry, and *Campylobacter* spp., *Salmonella*, and *Escherichia coli* have perhaps the greatest potential to impact public health. To conduct surveys of pathogen incidence and deduce meaningful information, proper methods must be employed during sample collection, transport, and processing. Nationwide surveys in particular require careful consideration of optimal sample transport conditions to ensure pathogen populations are unaffected during chilled transport of the sample from the field to central processing locations. Transport of the samples may take up to 24 h in some situations, and the samples must be maintained under refrigeration conditions, typically in coolers with artificial ice, to avoid changes in pathogen populations. Numerous carcass rinsing solutions have been proposed and utilized (2, 3, 8). Different carcass rinsing solutions may have an impact on pathogen survival during storage and transport because of differences in buffering capacity or other chemical and physical features. This study was conducted to compare the efficacy of sterile tap water (SW), buffered peptone water (BPW), and universal preenrichment broth (UP) for maintaining populations of *Campylobacter* spp., *Salmonella*, and *E. coli* for 24 h under simulated transport conditions. Because potable water differs in pH and hardness across the United States, a follow-up study was con-

ducted to investigate whether water hardness or pH within the ranges normally found across the United States would affect *Campylobacter* recovery from carcass rinses.

## MATERIALS AND METHODS

**Rinse comparison study.** Freshly processed broiler carcasses ( $n = 100$ ; postchill, postdrip) were procured from two commercial processing plants (50 carcasses each) and transported within 1 h on ice in waxed cardboard boxes to the Russell Research Center in Athens, GA, for microbiological analysis. Each carcass was removed separately from the box by technicians with clean latex gloves and was placed in a large plastic bag (37 by 52 cm; Cryovac, Duncan, SC). SW (400 ml) was added to each bag, and the carcass was shaken in a prescribed manner for 1 min (9). Approximately 300 to 350 ml of rinsate was retrieved from each carcass and kept on ice after collection. Each bottle of rinsate was then divided by pouring approximately 90 ml into three separate sample cups. These three subsamples were then slightly diluted by the addition of 10 ml of SW, 10× BPW, or 10× UP and were placed on ice. The BPW was prepared following the method of Juven et al. (4), and the UP was prepared as described by Bailey and Cox (1).

Preliminary studies indicated that low levels of naturally occurring *Salmonella* could be expected in the carcass rinses, so each sample was inoculated with  $7 \times 10^5$  cells of *Salmonella enterica* serotype Typhimurium (strain of avian origin resistant to >200 ppm of nalidixic acid) (6). Because naturally occurring *Campylobacter* spp. populations may be highly variable on carcasses, half of the samples (50) were inoculated with  $3.4 \times 10^4$  cells of a cocktail of three *Campylobacter* isolates also obtained

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TABLE 1. Recovery of target microbial populations from broiler carcasses rinsed with sterile water (SW), buffered peptone water (BPW), or universal preenrichment broth (UP) after 0 or 24 h of storage under simulated transport conditions

Carcass rinse	Mean (SD) bacterial populations in rinsate (log CFU/ml) <sup>a</sup>					
	Laboratory 1			Laboratory 2		
	0 h	24 h	P <sup>b</sup>	0 h	24 h	P <sup>b</sup>
Artificially inoculated with <i>Salmonella</i> Typhimurium (n = 100 carcasses)						
SW	3.29 (0.13) BC	3.47 (0.10) BC	<0.0001	3.42 (0.18) BC	3.49 (0.13) B	0.002
BPW	3.47 (0.10) A	3.52 (0.11) AC	0.0003	3.47 (0.09) A	3.44 (0.08) AC	0.0066
UP	3.49 (0.09) A	3.36 (0.12) AB	<0.0001	3.46 (0.10) A	3.52 (0.11) B	<0.0001
Naturally occurring <i>E. coli</i> (n = 100 carcasses)						
SW	1.02 (0.36) BC	0.94 (0.37) C	0.09	1.08 (0.41) BC	1.01 (0.39) BC	0.21
BPW	0.78 (0.46) AC	0.84 (0.43) C	0.38	0.92 (0.42) AC	0.87 (0.41) AC	0.47
UP	0.36 (0.39) AB	0.60 (0.46) AB	<0.0001	0.41 (0.48) AB	0.45 (0.44) AB	0.47
Naturally occurring <i>Campylobacter</i> (n = 50 carcasses)						
SW	1.35 (0.93) C	1.54 (0.65) BC	0.25	0.68 (0.58) B	0.91 (0.88) C	0.12
BPW	1.22 (0.71) C	1.00 (0.71) AC	0.13	1.27 (0.69) AC	1.24 (0.85) C	0.84
UP	0.52 (0.73) AB	0.42 (0.63) AB	0.50	0.55 (0.73) B	0.49 (0.65) AB	0.67
Artificially inoculated with <i>C. jejuni</i> (n = 50 carcasses)						
SW	2.37 (0.33) BC	2.54 (0.25) BC	0.0048	2.64 (0.28) BC	2.38 (0.29) BC	<0.0001
BPW	2.86 (0.17) A	2.74 (0.32) A	0.02	2.85 (0.19) A	2.88 (0.16) AC	0.50
UP	2.80 (0.17) A	2.79 (0.13) A	0.80	2.81 (0.14) A	2.74 (0.43) AB	0.29

<sup>a</sup> For each target population, values with different letters (A, SW; B, BPW; C, UP) indicate that recovery was significantly different from that with other media based on results of the Kolmogorov-Smirnov analysis of variance ( $P < 0.05$ ).

<sup>b</sup> P values for results of Tukey's *t* test comparing 0-h and 24-h samples by laboratory and carcass rinse medium.

from poultry samples. A 1-ml portion of each inoculum was added to the carcass rinse samples. Based on previously collected data (not shown), we anticipated uniform presence of *E. coli*, so this bacterium was not added to the samples. All samples were held for less than 3 h on ice and assayed to obtain an initial time 0 estimation of the levels of the three selected populations. Two individual laboratories duplicated the microbiological assays. Standard serial dilutions for direct plating on selective agars were prepared using phosphate buffered saline.

*Salmonella* Typhimurium populations were determined by direct plating of  $10^{-1}$  and  $10^{-2}$  dilutions on brilliant green sulfite agar with 200 ppm of nalidixic acid. These plates were incubated at 37°C for 24 h before typical colonies were counted. Because a marker strain of *Salmonella* Typhimurium resistant to nalidixic acid was used, extensive confirmation techniques were not necessary. Nevertheless, typical colonies from a small number of randomly selected plates were immunologically confirmed using group B antisera (Becton Dickinson, Sparks, MD).

*Campylobacter* populations were determined by direct plating of  $10^{-1}$  dilutions on Campy-cefex agar (10). The Campy-cefex plates were incubated at 42°C for 48 h under microaerobic conditions (10% CO<sub>2</sub>, 5% O<sub>2</sub>, and 85% N<sub>2</sub>). Several of the suspect *Campylobacter* colonies from inoculated samples were confirmed by latex agglutination (Integrated Diagnostics, Inc., Baltimore, MD). Approximately 90% of the naturally contaminated sample plates had *Campylobacter* colonies confirmed by latex agglutination, 25% of these colonies were further confirmed by microscopic observation of typical cellular morphology and motility after being restreaked onto Campy-cefex plates.

*E. coli* populations were determined by plating  $10^0$  and  $10^{-1}$  dilutions on Petrifilm (3M Microbiology, St. Paul, MN), which was incubated at 35°C for 24 h.

After the initial time 0 sampling, all samples were packed into insulated shipping containers and maintained at refrigeration

temperatures (4 to 8°C) with artificial ice packs. After 24 h of storage, the individual sample containers were again independently assayed for the three target organisms by personnel from the two laboratories as previously described. The data were collected and subjected to statistical analysis (Tukey *t* test and Kolmogorov-Smirnov analysis of variance) using StatMost for Windows (Dataxiom Software, Inc., Los Angeles, CA).

**Water hardness and pH study.** Because water hardness and pH differ across the United States, an experiment was conducted to assess whether hard water (136 ppm of CaCl<sub>2</sub>) or soft water (36 ppm of CaCl<sub>2</sub>) and pH variation (pH 6.5 or 7.5) adversely affects enumeration of *Campylobacter* spp. The different water types were prepared by the addition of CaCl<sub>2</sub> or HCl as appropriate. An additional 50 freshly processed broiler carcasses were obtained from a commercial processor and transported on ice to the laboratory. Ten carcasses were rinsed in 400 ml each of five water types: control, hard acidic, hard basic, soft acidic, and soft basic. Initial *Campylobacter* populations were enumerated as before. The samples were stored for 24 h at refrigeration temperatures, *Campylobacter* populations were determined, and data were subjected to statistical analysis again by methods as described above.

## RESULTS AND DISCUSSION

**Rinse comparison study.** Data from the 100-carcass comparison experiment are shown in Table 1. Recovery of *Campylobacter* spp., *E. coli*, and *Salmonella* Typhimurium from the broiler chicken carcass rinsates was very consistent between the two laboratories. The differences that were observed were not considered to be of major microbiological significance. Overall, there was an insignificant loss in cell numbers during the 24-h simulated transport period for

each of the target bacteria sampled. There was a very limited difference between levels of the three target bacteria detected after use of SW and BPW as transport media except for the *E. coli* counts. There appeared to be a slightly diminished recovery of *E. coli* when UP was used as the transport medium.

Tukey's analysis revealed few significant differences ( $P > 0.05$ ) in numbers of *E. coli* or *Campylobacter* spp. before or after simulated shipping or between types of rinse-transport media for recovery of *Campylobacter* spp. and *Salmonella*. The only significant difference discerned for the different transport media occurred when using UP for *E. coli* enumeration (Table 1). This transport medium recovered fewer *E. coli* cells than did either SW or BPW. Significant differences were observed in the number of *Salmonella* cells before and after transport for the two laboratories, but one laboratory obtained a slightly higher number and the other obtained a slightly lower number. From a practical standpoint, these differences of only a few cells among several thousands are microbiologically insignificant.

*Campylobacter* spp. are not capable of proliferating at room or chilled temperatures (5, 7). Therefore, when significance ( $P < 0.05$ ) is ascribed to increases in cell numbers, these differences are more likely due to biological variation or uneven distribution on the sample than to true microbiologically significant increases. For example, one laboratory found a slight but significant reduction in numbers of *Campylobacter* cells when SW was used as the transport medium, whereas the second laboratory found a minor increase in numbers of *Campylobacter* cells in a similar sample type (Table 1). These differences are also microbiologically insignificant, although because of the large number of samples and the very limited variation between samples within groups, the differences were considered statistically significant. Approximately 1-log higher levels of *Campylobacter* spp. were recovered from the artificially inoculated carcasses than on the naturally contaminated carcasses. Half (50) of the carcasses were inoculated with *Campylobacter* spp. because the naturally occurring levels of these organisms can be unevenly distributed on this sample type. Fortunately, in this case the carcasses were naturally contaminated, and we suggest that this information should be considered more important. The naturally occurring *Campylobacter* spp. had substantially reduced numbers only with the UP rinse solution.

Overall, these data are a good example of a situation in which low variation between samples can lead to statistical differences that are not microbiologically meaningful. Careful interpretation and microbiological experience should be used to discern truly important differences. Our opinion of these findings support the conclusion that either SW or BPW should be used as a broiler carcass rinse and/or transport medium to accurately reflect the levels or presence of these three target bacteria as a whole.

**Water hardness and pH study.** Results of the water hardness and pH study (Table 2) indicate that differences in pH and calcium ion concentration do not significantly

TABLE 2. Assessment of effect of water hardness and pH on *Campylobacter* recovery from broiler carcasses ( $n = 25$ ) rinsed with sterile water before and after 24-h simulated transport

Water type	CaCl <sub>2</sub> (ppm)	pH	Mean (SD) <i>Campylobacter</i> populations in rinsate (log CFU/ml) <sup>a</sup>	
			0 h	24 h
Control	38	7.5	1.37 (0.23)	1.04 (0.35)
Hard acidic	136	6.5	1.29 (0.19)	1.32 (0.19)
Hard basic	136	7.5	1.56 (0.22)	1.28 (0.21)
Soft acidic	36	6.5	1.17 (0.33)	0.93 (0.53)
Soft basic	36	7.5	1.40 (0.26)	1.23 (0.19)

<sup>a</sup> Differences between populations recovered in different water types were not significant based on results of the Kolomogorov-Smirnov analysis of variance ( $P > 0.05$ ).

affect ( $P > 0.05$ ) the ability of different potable water types commonly found in the United States to recover campylobacters from carcasses in rinse procedures either before or after a 24-h simulated shipping time. Because *Campylobacter* spp. are thought to be the most environmentally fragile of the three target organisms (9), it might be assumed that both *E. coli* and *Salmonella* would likewise survive transport in the various potable waters found within the United States.

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